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Prevalence of *Toxoplasma gondii* from Free-Range Chickens (*Gallus domesticus*) from Addis Ababa, Ethiopia

G. Tilahun, N. Tiao*, L. R. Ferreira†, S. Choudhary†, S. Oliveira†, S. K. Verma†, O. C. H. Kwok†, B. Molla*, W. J. A. Saville*, G. Medhin, T. Kassa, H. Aleme, W. A. Gebreyes*, C. Su†, and J. P. Dubey‡, Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia; *Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio 43210-1092; †Department of Microbiology, The University of Tennessee, Knoxville, Tennessee 37996-0845; ‡United States Department of Agriculture, Agricultural Research Service, Beltsville Agricultural Research Center, Animal Parasitic Diseases Laboratory, Building 1001, Beltsville, Maryland 20705-2350. Correspondence should be sent to: *jitender.dubey@ars.usda.gov*

ABSTRACT: Prevalence of *Toxoplasma gondii* in free-range chickens (*Gallus domesticus*) is a good indicator of the environmental contamination with oocysts because chickens become infected mainly by feeding from ground, feed, or soil contaminated with oocysts. The seroprevalence of *T. gondii* antibodies in 125 free-range chickens from the Addis Ababa, Ethiopia, was determined. Antibodies to *T. gondii* were assayed by the modified agglutination test; 48 of 125 (38.4%) chickens were seropositive, with titers of 1:5 in 14, 1:10 in 12, 1:20 in 14, 1: 40 in 3, 1: 80 in 1, 1:160 in 1, 1:320 in 1, and ≥1:640 in 2 chickens. The hearts of 115 chickens were bioassayed for *T. gondii* infection. Hearts of 72 seronegative (modified agglutination test [MAT] < 1:5) chickens were pooled in 4 groups (20 + 18 + 19 + 15) and fed to 4 *T. gondii*-free cats; none of these 4 cats shed oocysts in their feces examined 3–21 days after feeding chicken tissues. Hearts of 43 seropositive chickens (MAT ≥ 1:5) were bioassayed individually in mice. *Toxoplasma gondii* was isolated from only 1 chicken, with a MAT titer of 1:80. This isolate was designated TgCKEt1 and was not pathogenic for outbred mice. Restricted fragment length polymorphism (RFLP) genotyping using 10 loci indicated the TgCKEt1 was ToxoDB polymerase chain reaction-RFLP genotype #1 (Type II clonal). Results of this study indicate very low environmental contamination with *T. gondii* oocysts around Addis Ababa.

Toxoplasma gondii infections are widely prevalent in humans and animals worldwide (Dubey, 2010a). Humans become infected post-natally by ingesting tissue cysts from undercooked meat, consuming food or drink contaminated with oocysts, or by accidentally ingesting oocysts from the environment. Felids are the most important host in the life cycle of *T. gondii* because they excrete environmentally resistant oocysts. Cats become infected with *T. gondii* by eating infected tissues from intermediate hosts. Among intermediate hosts, infected birds and rodents are considered the most important sources of *T. gondii* infection for cats.

Toxoplasma gondii infection in free-range (FR) chickens (*Gallus domesticus*) is considered important because FR chickens are one of the best indicators for soil contamination with *T. gondii* oocysts because they feed from the ground or in soil. Tissues of infected chickens are considered a good source of infection for cats, humans, and other animals (Ruiz and Frenkel, 1980; Dubey, 2010a). Rarely, *T. gondii* can cause clinical disease in chickens (Dubey, 2010b).

Limited data indicate a high seroprevalence of *T. gondii* antibodies in humans and animals in Ethiopia, but little is known of clinical toxoplasmosis in humans and animals (Dubey et al., 2012). Much medical attention is being focused on the acquired immune deficiency syndrome (AIDS) epidemic in Africa. Ethiopia is the second-most populous nation in Africa, with >82 million inhabitants, and a high rate of human immunodeficiency virus (HIV)/AIDS. Clinical toxoplasmosis in untreated HIV/AIDS patients in Ethiopia is of public health concern (Dubey et al., 2012). In a preliminary study, antibodies to *T. gondii* were detected in 85.4% of 48 stray cats from Addis Ababa, Ethiopia, and viable *T. gondii* was isolated from the hearts of 26 of 36 cats bioassayed (Dubey et al., 2013; Tiao et al., 2013). In addition, *T. gondii* oocysts were found in feces of 7 of 36 cats (Dubey et al., 2013). Here, we report seroprevalence of *T. gondii* from FR chickens from the Addis Ababa area.

In June 2012, backyard local species of chickens were purchased from the 10 districts of Addis Ababa, Ethiopia (Table I). The chickens were purchased, killed, and then blood was collected. Serum and hearts were separated and kept in 4 C and transported first to Columbus, Ohio, and then to the Animal Parasitic Diseases Laboratory (APDL), United States Department of Agriculture (USDA), Beltsville, Maryland, for *T. gondii* examination. Ten days elapsed between killing of chickens and receipt of chickens at APDL. Inadvertently, samples were transported partly by road and partly by air from Columbus to Beltsville. Chickens were handled following the Animal Care Protocol approved by Institutional Review Board of Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia.

Sera of chickens were tested for *T. gondii* antibodies using the modified agglutination test (MAT) as described by Dubey and Desmonts (1987). Initially, sera were screened at 1:5, 1:10, 1:20, and 1:40 dilutions; subsequently seropositive sera were diluted to 1:640 to test for *T. gondii* antibodies.

Of these 125 chickens, hearts of 115 chickens were available for bioassay for *T. gondii* infection. Hearts of 72 seronegative (MAT < 1:5) chickens were pooled in 4 groups (20 + 18 + 19 + 15) and fed to 4 *T. gondii*–free cats. Feces of the cat were examined 3–21 days after feeding chicken tissues for *T. gondii* oocysts, as described previously (Dubey, 2010b). Hearts of 43 seropositive chickens (MAT ≥ 1:5) were bioassayed individually in mice. The entire heart was homogenized in saline, digested in pepsin, and bioassayed in 2 Swiss Webster (SW) mice and 1 γ-interferon gene knockout (KO) mouse for *T. gondii* infection (Dubey, 2010a). The inoculated mice were examined for *T. gondii* infection as described previously (Dubey, 2010a); they were considered infected with *T. gondii* when tachyzoites or tissue cysts were found in tissues. *Toxoplasma gondii* obtained in mice were cultivated in African green monkey cells (CV1). For this cultivation, homogenate of infected mouse lungs was homogenized in saline with antibiotics, and the homogenate was seeded onto cell cultures. Tachyzoites were harvested from cell cultures, cryopreserved, and processed for DNA extraction.

Antibodies to *T. gondii* were found in 48 of 125 (38.4%) chickens (Table I). Viable *T. gondii* was isolated from the heart of chicken 69, with a MAT titer of 1:80. Of the 3 mice inoculated with heart of chicken 69, the 2 SW mice developed MAT antibodies, and *T. gondii* tissue cysts were found in their brains when killed 38 days post-inoculation (PI); both mice remained asymptomatic. The third mouse (KO mouse) remained uninfected. Two KO mice sub-inoculated with homogenate of brains of the infected SW mice became ill, and tachyzoites were found in their lungs when killed 17 and 22 days PI. Tachyzoites were propagated in cell cultures seeded with tachyzoites from lungs of inoculated mice. None of the 4 cats fed hearts of seronegative chickens shed oocysts in their feces.

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TABLE I. Seroprevalence of *Toxoplasma gondii* antibodies in free-range chickens from 10 districts in Addis Ababa, Ethiopia.

	Chickens		No. of chickens with titers of							
District	No. tested	No. seropositive (%)	5	10	20	40	80	160	320	≥640
Addis Ketema	15	5 (33.3)	1	1	3					
Akaki Kaliti	12	4 (33.3)	2		2					
Arada	7	1 (14.2)	1							
Bole	8	1 (12.5)		1						
Gullele	9	4 (44.4)	2		2					
Kirkos	3	1 (33.3)		1						
Kolfe-Keranio	21	11 (52.3)	3	4	4					
Lideta	21	10 (47.6)	4	1	1	2	1		1	
Nifas Silk-Lafto	11	3 (27.2)		1	2					
Yeka	18	8 (44)	1	3		1		1		2
Total	125	48 (38.4)	14	12	14	3	1	1	1	2

Toxoplasma gondii DNA was extracted from the tissues of cell culture–derived tachyzoites. Strain typing was performed using the genetic markers SAG1, 5′- and 3′-SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, alt.SAG2, and Apico, as described previously (Su et al., 2010). Results indicated that the chicken isolate was ToxoDB#1 (Type II clonal), most prevalent in Europe and North America (Su et al., 2012).

The MAT has been widely used to determine seroprevalence of *T. gondii* in chickens (Dubey, 2010b). Although the cut-off titer that should be considered specific for the diagnosis of *T. gondii* infection in chickens has not been determined, viable *T. gondii* has been isolated from 17.4% of 103 FR chickens with a MAT titer of 1:5 (J.P.D., unpublished). Therefore, we provided all MAT titers in Table I. A recent review (Dubey, 2010b) provided ample evidence that chickens raised not only in back yard operations but also in large commercial free-range operations harbor viable *T. gondii* (Dubey, 2010b). In many instances, especially in developing countries, these chickens are killed at home or in unsupervised slaughter facilities and the viscera are left for scavengers or are improperly disposed off. *Toxoplasma gondii* infection can be transmitted if care is not taken to wash hands thoroughly after cutting meat and during cooking of meat; however, risk assessment studies have not been undertaken.

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LITERATURE CITED

DUBEY, J. P. 2010a. Toxoplasmosis of animals and humans, 2nd ed. CRC Press, Boca Raton, Florida, 313 p.

———. 2010b. *Toxoplasma gondii* infections in chickens (*Gallus domesticus*): Prevalence, clinical disease, diagnosis, and public health significance. Zoonoses and Public Health **57:** 60–73.

———, AND G. DESMONTS. 1987. Serological responses of equids fed *Toxoplasma gondii* oocysts. Equine Veterinary Journal **19:** 337–339.

———, C. DARRINGTON, N. TIAO, L. R. FERREIRA, S. CHOUDHARY, B. MOLLA, W. J. A. SAVILLE, G. TILAHUN, O. C. H. KWOK, AND W. A. GEBREYES. 2013. Isolation of viable *Toxoplasma gondii* from tissues and feces of cats from Addis Ababa, Ethiopia. Journal of Parasitology **99:** 56–58.

———, N. TIAO, W. A. GEBREYES, AND J. L. JONES. 2012. A review of toxoplasmosis in humans and other animals in Ethiopia. Epidemiology and Infection **140:** 1935–1938.

RUIZ, A., AND J. K. FRENKEL. 1980. Intermediate and transport hosts of *Toxoplasma gondii* in Costa Rica. American Journal of Tropical Medicine and Hygiene **29:** 1161–1166.

SU, C., A. KHAN, P. ZHOU, D. MAJUMDAR, D. AJZENBERG, M. L. DARDÉ, X. Q. ZHU, J. W. AJIOKA, B. M. ROSENTHAL, J. P. DUBEY, AND L. D. SIBLEY. 2012. Globally diverse *Toxoplasma gondii* isolates comprise six major clades originating from a small number of distinct ancestral lineages. Proceedings of the National Academy of Sciences U.S.A. **109:** 5844–5849.

———, E. K. SHWAB, P. ZHOU, X. Q. ZHU, AND J. P. DUBEY. 2010. Moving towards an integrated approach to molecular detection and identification of *Toxoplasma gondii*. Parasitology **137:** 1–11.

TIAO, N., C. DARRINGTON, B. MOLLA, W. J. A. SAVILLE, G. TILAHUN, O. C. H. KWOK, W. A. GEBREYES, M. R. LAPPIN, J. L. JONES, AND J. P. DUBEY. 2013. An investigation into the seroprevalence of *Toxoplasma gondii, Bartonella* spp., feline immunodeficiency virus (FIV), and feline leukemia virus (FeLV) in cats in Addis Ababa, Ethiopia. Epidemiology and Infection **141:** 1029–1033.